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Saffold Cardioviruses in Children with Diarrhea, Thailand

To the Editor: Cardioviruses currently consist of at least 3 viruses: Theiler murine encephalomyocarditis virus, encephalomyocarditis virus, and Saffold virus (SAFV) (*1*–*4*). Saffold cardiovirus in the family *Picornaviridae* was isolated and identified from fecal specimens of a child with fever of unknown origin in the United States (*3*).

Several reports have documented the presence of SAFV in fecal samples and respiratory secretions (*5*–*10*). However, it is not clear whether SAFV is associated with any disease, including gastroenteritis in humans, and epidemiologic data for SAFV are limited. We report an epidemiologic survey of SAFV in children hospitalized with diarrhea in Chiang Mai, Thailand.

A total of 150 fecal specimens were obtained from children hospitalized with acute gastroenteritis in Chiang Mai during January–December 2007. Patient ages ranged from >1 to 5 years. SAFV in fecal specimens was detected by using a nested PCR and primers specific for the virus 5′ untranslated region (*7*). A negative control was also included to monitor any contamination that might have occurred during the PCR.

SAFVs detected were further analyzed by amplification of the viral protein (VP) 1 gene (*6*,*9*,*10*) and direct sequencing of the VP1 PCR amplicon by using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). VP1 sequence was compared with VP1 sequences of reference strains available in the National Center for Biotechnology Information (Bethesda, MD, USA). Phylogenetic and molecular evolutionary analyses were conducted by using MEGA4 (www.megasoftware.net). Nucleotide sequences of SAFV strains described were deposited in GenBank under accession nos. HQ668170–HQ668173.

Four (2.7%) of 150 specimens were positive for SAFV (CMH023/2007, CMH038/2007, CMH045/2007, and CMH143/2007). Two of these specimens (CMH023/2007 and CMH038/2007) were obtained in February 2007, one (CMH045/2007) in March 2007, and 1 (CMH143/2007) in November 2007. Co-infections with other viruses were detected in all 4 samples. Two specimens (CMH023/2007and CMH045/2007), were co-infected with noroviruses GII/16 and GII/4 genotypes, respectively. One SAFV-positive sample (CMH038/2007) was co-infected with a group A rotavirus G1P[8] genotype, and another (CMH143/2007) was co-infected with human parechovirus.

All SAFV-positive specimens were further amplified for the VP1 gene to determine their phylogenetic lineages and genetic relationships with other SAFV reference strains. When we used 3 sets of primers used in other studies (*6*,*9*,*10*) for amplification of the VP1 gene, this gene was amplified only by the primer set reported by Itagaki et al. (*10*).

Analysis of partial VP1 sequences (369 nt) of 4 SAFV strains showed that strains CMH023/2007 and CMH143/2007 were highly conserved (nt sequence identities >97%). These 2 SAFV strains were most closely related to the prototype strain of SAFV1 (EF165067) isolated in the United States (nt sequence identity range 87.6%–88.9%) and SAFV strains from China (LZ50419, BCH895, GL311, and GL377) (Figure). In addition, the other 2 SAFVs identified in the present study (CMH038/2007 and CMH045/2007) were identical to each other and closely related to SAFV2 strains from China (BCHU79, BCHU353)

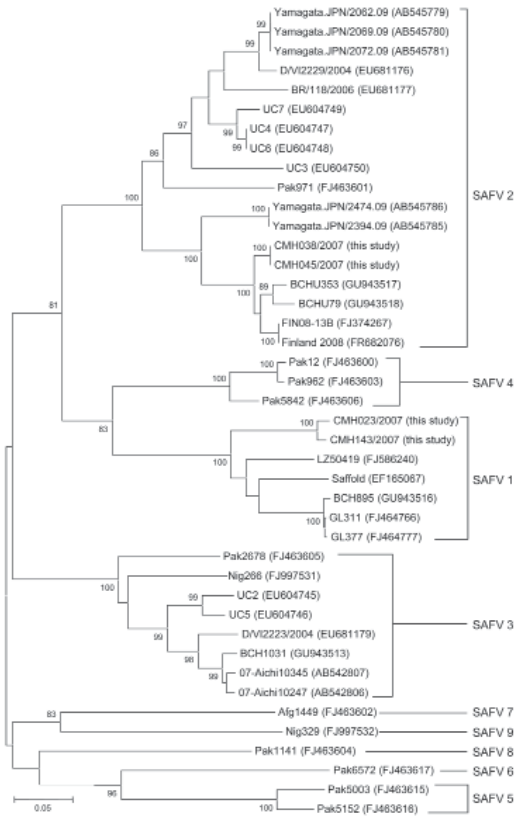

Figure. Phylogenetic analysis of the partial nucleotide sequence (369 nt) encoding the viral protein 1 gene of Saffold virus (SAFV) isolated in this study and other reference strains. The tree was generated by using the neighbor-joining method and MEGA4 (www.megasoftware.net). Bootstrap values >80 are indicated for the corresponding nodes on the basis of a resampling analysis of 1,000 replicates. Scale bar indicates nucleotide substitutions per site.

and Finland (Finland 2008, FIN08–13B) (nt sequence identity range 94.8%–95.6%). Phylogenetic analysis showed that CMH038/2007 and CMH045/2007 were clustered within the SAFV2 lineage (Figure).

The 4 strains of SAFV were isolated from children with acute gastroenteritis who were co-infected with other viral pathogens (norovirus, group A rotavirus, and human parechovirus). Therefore, we could not determine whether SAFVs identified in this study were associated with acute gastroenteritis. The detection rate for SAFV in children with acute gastroenteritis (2.7%) in our study was consistent with that in a study in Beijing, People's Republic of China (3.2%) (*9*).

Phylogenetic analysis of the VP1 region demonstrated that 2 SAFV lineages (SAFV1 and SAFV2) were circulating in Chiang Mai, Thailand. Further extensive epidemiologic surveillance of SAFV in other areas may provide a better understanding of the distribution, heterogeneity, and association of SAFV with enteric diseases in humans.

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Lethal Necrotizing Pneumonia Caused by an ST398 *Staphylococcus aureus* Strain

To the Editor: The prevalent colonization of livestock with methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type (ST) 398 in many countries is a cause for consternation. However, understanding of the emergence of these organisms and their public health implications is embryonic. The perceptions that all MRSA found in livestock are of ST398 lineage or that livestock are the only reservoirs of ST398 oversimplify a complex epidemiology, therefore, prudence is required when attributing human infections with *S. aureus* ST398 to livestock reservoirs. The fatal infection of a young girl with ST398 methicillin-susceptible *S. aureus* (MSSA) is tragic (*1*). However, the conclusion by the authors that "the spread of *S. aureus* ST398 among livestock is a matter of increasing concern because strains of this sequence type were able to acquire PVL [Panton-Valentine leukocidin] genes" is misleading.

The authors report no history of livestock exposure and the *spa* type reported (t571) is relatively rare among livestock isolates (*2*,*3*). The isolate from the fatal case was tetracycline-susceptible and positive for PVL toxin, while livestock ST398 isolates have been almost uniformly tetracycline resistant and PVL negative. Notably, *spa* type t571 ST398 MSSA was detected in 9 families from the Dominican Republic living in Manhattan, New York, without contact with livestock (*4*). Furthermore, t571 was the only *spa* type of MSSA identified in a study in the Netherlands of ST398 isolates, including 3 independent cases of nosocomial bacteremia in Rotterdam with no apparent livestock contact (*5*). *spa* type t571 was the predominant (11%) MSSA type in patients at a Beijing, China, hospital (*6*). More recently, a study of t571 MSSA strains from cases of bloodstream infections in France determined that the isolates differed from pig-borne strains and shared similarities with strains from humans in China and virulent USA300 strains (*7*). These observations concur with a hypothesis that ST398 strains of diverse genotype and geographic origin may also be epidemiologically distinct (*8*), and livestock contact is a notably inconsistent feature of invasive ST398 infections (*5*,*7*–*10*).

The possibility that variants of the ST398 lineage may persist in human populations without livestock contact should not be dismissed. The incidence and severity of clinical infections with ST398 *S. aureus* in livestock workers as yet have been minimal. Understanding the public health implications of ST398 *S. aureus* requires systematic investigation of their epidemiology in animals and humans. Human clinical cases of ST398 *S. aureus* infection should not be indiscriminately attributed to livestock, particularly if isolates are genotypically dissimilar to those occurring commonly in animals.

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